Clinica Chimica Acta 493 (2019) 156–161

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Evaluation of 99th percentile and reference change values of a high-sensitivity cTnI method: A multicenter study☆

Aldo Clerico[a,*], Andrea Ripoli[a], Silvia Masotti[a], Veronica Musetti[a], Rosalia Aloe[b], Mariella Dipalo[b], Sara Rizzardi[c], Ruggero Dittadi[d], Cinzia Carrozza[e], Simona Storti[a], Lucia Belloni[f], Marco Perrone[g], Tommaso Fasano[f], Simone Canovi[f], Mario Correale[h], Concetta Prontera[a], Cristina Guiotto[i], Domenico Cosseddu[i], Marco Migliardi[i], Sergio Bernardini[g]

[a] Laboratorio Clinico, Fondazione CNR-Regione Toscana, G. Monasterio e Scuola Superiore Sant'Anna, Pisa, Italy
[b] S. S. Dipartimentale Biochimica ad Elevata Automazione, Dipartimento Diagnostico Azienda Ospedaliero-Universitaria di Parma, Parma, Italy
[c] Azienda Socio-Sanitaria Territoriale di Cremona, UO Laboratorio analisi chimico cliniche e microbiologiche, Cremona, Italy
[d] UOC Medicina di Laboratorio, Ospedale dell'Angelo ULSS 3 Serenissima, Mestre, Italy
[e] UOC Chimica, Biochimica e Biologia Molecolare Clinica, Fondazione Policlinico Universitario "A. Gemelli", IRCCS, and Università Cattolica del Sacro Cuore, Roma, Italy
[f] Laboratorio di Autoimmunità, Allergologia e Biotecnologie Innovative, Azienda Unità Sanitaria Locale, IRCCS and Laboratorio Analisi Chimico-Cliniche ed Endocrinologia-ASMN, Azienda Ospedaliera di Reggio Emilia, Italy
[g] Laboratorio Analisi Cliniche, Azienda Ospedaliera Universitaria Tor Vergata, Roma, Italy
[h] Unità di Patologia Clinica, IRCCS De Bellis, Castellana Grotte, e Laboratorio di Analisi Cliniche e Microbiologiche P.O. San Paolo – ASL Bari, Bari, Italy
[i] S.C. Laboratorio Analisi, A.O. Ordine Mauriziano di Torino, Torino, Italy

ARTICLE INFO

Keywords:
Cardiac troponins
Reference population
High-sensitivity methods
Risk stratification
Acute coronary syndrome
Myocardial infarction

ABSTRACT

Background: The Italian Society of Clinical Biochemistry (SIBioC) and the Italian Section of the European Ligand Assay Society (ELAS) have recently promoted a multicenter study (Italian hs-cTnI Study) with the aim to accurately evaluate analytical performances and reference values of the most popular cTnI methods commercially available in Italy. The aim of this article is to report the results of the Italian hs-cTnI Study concerning the evaluation of the 99th percentile URL and reference change (RCV) values around the 99th URL of the Access cTnI method.

Materials and methods: Heparinized plasma samples were collected from 1306 healthy adult volunteers by 8 Italian clinical centers. Every center collected from 50 to 150 plasma samples from healthy adult subjects. All volunteers denied the presence of chronic or acute diseases and had normal values of routine laboratory tests (including creatinine, electrolytes, glucose and blood counts). An older cohort of 457 adult subjects (mean age 63.0 years; SD 8.1 years, minimum 47 years, maximum 86 years) underwent also ECG and cardiac imaging analysis in order to exclude the presence of asymptomatic cardiac disease.

Results and conclusions: The results of the present study confirm that the Access hsTnI method using the DxI platform satisfies the two criteria required by international guidelines for high-sensitivity methods for cTn assay. Furthermore, the results of this study confirm that the calculation of the 99th percentile URL values are greatly affected not only by age and sex of the reference population, but also by the statistical approach used for calculation of cTnI distribution parameters.

1. Introduction

Cardiac troponin I (cTnI) and T (cTnT) are the preferred biomarker for the differential diagnosis of acute coronary syndrome (ACS) [1–3]. International guidelines recommended that the increase in cTnI or cTnT levels over the 99th percentile upper reference limit (99th URL) should be considered as clinically relevant, and they also specifically indicated that this cut off value should be measured with an imprecision ≤10 CV % [1–3]. Only after the year 2006, some manufacturers set-up the first new generation of cTnI and cTnT immunoassays with improved

☆ Study promoted by the Italian Society of Clinical Biochemistry (SIBioC) and the Italian Section of the European Ligand Assay Society (ELAS).

* Corresponding author at: Laboratory of Cardiovascular Endocrinology and Cell Biology, Department of Laboratory Medicine, Fondazione CNR Toscana G. Monasterio, Scuola Superiore Sant'Anna, Via Trieste 41, 56126 Pisa, Italy.
E-mail address: clerico@ifc.cnr.it (A. Clerico).

https://doi.org/10.1016/j.cca.2019.02.029
Received 11 February 2019; Accepted 27 February 2019
Available online 28 February 2019

analytical sensitivity with the aim to satisfy the quality specifications requested by international guidelines and consensus documents [4–14]. The 2018 Expert Opinion from the AACC and IFCC [2] recommends that high-sensitivity methods for cTnI and cTnT assay should satisfy two fundamental criteria. First, high-sensitivity methods should measure the 99th percentile URL with an imprecision (expressed as CV %) ≤10%. Second, high-sensitivity methods should measure cTn concentrations above the LoD of the method at least in 50% healthy subjects enrolled in large populations including > 300 men and 300 women [2]. The 99th URL values not only strongly depend on demographic and physiological variables of populations (i.e., the criteria for considering the reference population "healthy"), but also on the analytical performances of cTnI methods, as well as on the mathematical algorithms used for calculating the 99th percentile URL [15,16].

In 2016, the guidelines of European Society of Cardiology (ESC) on the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation (NSTEMI) suggested 0 h/1 h rule-in and rule-out algorithms when measurement of cardiac troponins is performed using high-sensitivity immunoassay methods [1]. These guidelines recommend the use of absolute change (i.e., expressed as difference in cTn concentrations, ng/L) rather than percentage variation for the assessment of the rise and/or fall of cTn values [1].

According to the claims of international guidelines [1–3], the Italian Society of Clinical Biochemistry (SIBioC) and the Italian Section of the European Ligand Assay Society (ELAS) have recently promoted a multicenter study (named Italian hs-cTnI Study) with the aim to accurately evaluate analytical performances and reference values of the most popular cTnI methods commercially available in Italy. The most important aims of this article are to report and discuss the results of the Italian hs-cTnI Study concerning the evaluation of the 99th percentile URL and reference change (RCV) values around the 99th URL of the Access method, for cTnI assay using UniCell DxI 800 automated platform (named Access hsTnI, REF B52699), recently distributed to the Italian clinical laboratories.

2. Materials and methods

2.1. Reference population and plasma sample collection

The Italian hs-cTnI Study is a multicenter clinical study. Heparinized plasma samples were collected from a total of 1306 healthy adult volunteers by 8 Italian clinical centers, including both University and Regional Hospitals, which have highly qualified workforce staff in emergency, cardiology and laboratory departments. Every center collected from 50 to 150 plasma samples from healthy adult subjects enrolled from the clinical and laboratory staff or volunteers blood donors with age from 18 to 86 years.

All volunteers denied the presence of chronic or acute diseases and had normal values of routine laboratory tests (including creatinine, electrolytes, glucose and blood counts) [17]. In particular, to more accurately evaluate cTnI concentrations of healthy subjects older than 47 years, plasma samples from 457 adult subjects collected in the MEHLP study were also assayed (mean age 63.0 years; SD 8.1 years, minimum 47 years, maximum 85 years). The MEHLP study is a screening study aimed to evaluate the cardiovascular subclinical disease in an asymptomatic general population with age > 45 years from the community of Montignoso (Massa, Italy), as reported in detail elsewhere [17]. All the subjects enrolled in the MEHLP study underwent an accurate health investigation by means of a thorough clinical examination and routine laboratory tests [17]. Furthermore, lifestyle habits and medical history were collected by questionnaires. Participants to the MHELP study underwent also ECG and cardiac imaging analysis (computed tomography scan, carotid echography, echocardiography). Exclusion criteria were: presence of cardiac or systemic acute or chronic diseases, such as myocardial infarction, heart failure, coronary heart disease, hypertension, diabetes, kidney disease, obesity, tumour, hepatitis, BPCO, and use of drugs [17].

Every laboratory participating to the study stored at −80 °C two aliquots of about 1 mL of plasma samples collected from healthy subjects in tubes identified by alphanumeric barcodes. The stored tubes were sent to the reference laboratory of the study (Fondazione CNR Regione Toscana G. Monasterio, Pisa Italy) using a pack with dry ice within one months after the blood collection. Only age and the sex of adult healthy volunteers were known by the staff of the reference laboratory. In the reference laboratory the clinical samples were immediately stored at −80° and then the samples were measured within three months with the Access cTnI immunoassay.

The informed consent was obtained by all subjects enrolled in the study in accordance with the guidelines recommended by the respective local ethical committees.

2.2. cTnI immunoassay method

The Access hsTnI (REF B52699) assay is a two-site immunometric assay method (Beckman Coulter, Inc. Brea, CA 92821 USA). In the reference laboratory of the Italian hs-cTnI Study were previously evaluated the analytical characteristics and performances of the Access cTnI method [11]. The plasma samples were assayed in the reference laboratory according to the analytical procedures suggested by the manufacture using the DxI 800 platform [12]. Limits of blank (LoB), detection (LoD), and quantitation (LoQ) at 10% CV and 20% CV were calculated according to international standardized protocols [18,19], as previously described in details [11]. The imprecision profile was estimated by repeatedly measuring 10 heparinized plasma pools collected from healthy subjects and patients with cardiovascular diseases, as previously reported in detail [11]. These pools with mean cTnI concentrations ranging from about 1 ng/L to about 50 ng/L were repeatedly measured in 40 different runs with at least 3 different calibrations using two or more lots of reagents throughout two months. To calculate the limit of quantification (LoQ), the relationship between the error of the measurement (expressed as CV values, Y-axis) and cTnI concentrations (X-axis) was interpolated by means of a nonlinear regression curve [11].

The Reference Change Values (RCV) were evaluated according to Callum G. Fraser [20]. The bidirectional *Z*-score RCV between two results (CI 95%) were calculated by considering both the analytical variability of the method (CV_A) and the intra-individual variability (CV_I), using the formula: $RCV = 1.96\ [2(CV_A\ [2]\ +\ CV_I^2)]^{1/2}$.

2.3. Statistical Analysis

For the evaluation and comparison of the analytical performance of tested cTnI immunoassay methods, standard statistical analyses were carried out using the JMP program (version 12.1.0, SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). As cTnI circulating levels are not normally distributed, both non-parametric and parametric tests after logarithmic transformation (log-10) of data were used for statistical analysis. For the calculation of mean and standard deviation (SD) only robust methods were used. The identification of outlier values was performed by means of the based on the interquartile range (IQR) assuming a log-normal distribution [21]. An outlier value was evaluated by the formula: cTnI > Q_3 + 3 IQR, as gating parameter; where, Q_3 and IQR respectively are the third quartile and interquartile range ($Q_3 - Q_1$) of cTnI distribution.

The calculation of cTnI distribution and 99th percentile URL values was performed with the JMP program using nonparametric method (Fig. 1A), as recommended by international guidelines [2]. Lognormal distribution using a robust method was also calculated for comparison (Fig. 1B). The 99th percentile and the respective confidence interval (CI) values (at 90% and 95%) were also calculated with adjusted bootstrap percentile method according to Carpenter & Bithelll using

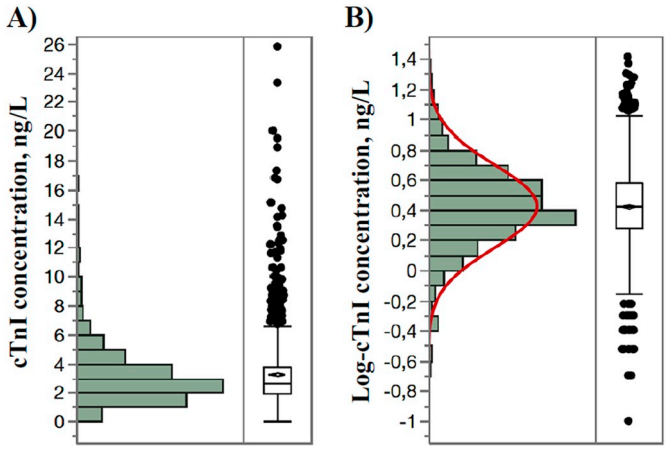

Fig. 1. Distribution of cTnI concentration value in 1302 healthy subjects.
Part A – Distribution of original cTnI values.
Part B – Distribution of log_{10}-transformed cTnI values. The log-normal distribution is indicated in red color.
In the figure are also reported the median, the interquartile range (i.e., 25th and 75th percentiles as box), and the 10th and 90th percentiles. The values below the 10th percentile and above the 90th percentile are reported with black circles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

random replacement and 100,000 repetitions [22].

3. Results

3.1. Analytical parameters and RCV of cTnI immunoassays

The analytical parameters of the Access cTnI method evaluated by the reference laboratory of the study using international standardized protocols [18,19] are reported in Table 1. For comparison, analytical parameter values, suggested by manufacturer and two previous studies, are also reported in Table 1 [23,24].

The RCV and absolute critical change (Δ change) values of the Access cTnI method using DxI 800 platform in the range of cTnI concentrations from 2 ng/L to 40 ng/L are reported in Table 2. These change values were calculated assuming an intra-individual variability (CV_I) in healthy adult subjects of 9%, according to Van der Linden et al. [25]. As indicated by the imprecision profile previously reported [11], the analytical variability per cTnI values ≥40 ng/L can be assumed to be constant and equal to about 5%, and so the RCV 95% and 99% CI are also constant and equal to about 29% and 38%, respectively.

3.2. cTnI distribution values of reference population

The total population with measured cTnI concentrations included 1306 individuals. According to the result of Tukey's test, the cTnI values of 4 individuals (2 men and 2 women) were excluded as outliers from the reference population. As a result, the population evaluated for the calculation of cTnI reference distribution parameters consisted of 1302 healthy adults subjects (age range from 18 to 86 years), including 650 women (mean age 51.7 years, SD 14.5 years) and 652 men (mean age 51.1 years, SD 13.9 years), well matched for age ($P = .4849$) and sex-ratio (F/M = 0.997). The distribution of cTnI values of the reference population was highly skewed (Fig. 1A), while log-transformed cTnI values roughly approximated a log-normal distribution (Fig. 1B). Mean, median and percentile values of cTnI values of the reference population, evaluated by nonparametric method, are also reported in Table 3. The values of 99th percentiles, calculated with bootstrap method were also reported in Table 3. On average, women showed significantly lower cTnI values than men ($p < .0001$ by Wilcoxon/Kruskal-Walls test). The distribution parameters, respectively found in men and women, are also reported in Table 3. Of note, 558 women (of 650, corresponding to 85.8%) had cTnI concentrations equal or higher than the LoD value (i.e., 1.3 ng/L), evaluated in the present study (Table 1). Furthermore, the median of the cTnI values of women is 2.2 ng/L (Table 3), which is the value of LoD value reported by the manufacturer (Table 1). The number of men with cTnI ≥1.3 ng/L (i.e., the LoD value evaluated in the present study) was 625 (of 652) corresponding to 95.8%.

Table 1
Analytical parameters of the Access cTnI method.

	LoB (ng/L)	LoD (ng/L)	LoQ 20% CV (ng/L)	LoQ 10% CV (ng/L)	References
Masotti et al.	0.6	1.3	2.1	5.3	[11]
Lippi et al.	0.14	0.34	–	1.35	[23]
Pretorius et al.	0.3	0.6	–	–	[24]
Manufacturer	1.7	2.3	2.3	5.6	Technical Bulletin

Table 2
Reference Change Value (RCV) and absolute critical change (Δ change) values of the Access cTnI method using DxI 800 platform in the range of cTnI concentration from 2 ng/L to 40 ng/L.

cTnI concentration (ng/L)	CV_A (%)	RCV 95% CI (%)	Δ change 95% CI (ng/L)	RCV 99% CI (%)	Δ change 99% CI (ng/L)
2	21.1	63.6	1.3	83.6	1.7
5	10.4	38.1	1.9	50.1	2.5
10	6.8	31.3	3.1	41.1	4.1
15	5.6	29.4	4.4	38.6	5.8
20	5.1	28.7	5.7	37.7	7.5
40	4.2	27.5	11.0	36.2	14.5

CV_A: Analytical variability evaluated according to the imprecision profile, as previously reported [11].
RCV 95% and 99%: Reference Change Values calculated at the probability of 95% and 99% CI, respectively.
Δ Change 95% and 99%: Absolute critical change calculated at the probability of 95% and 99%, respectively.

3.3. Influence of age and sex on cTnI values in the reference population

Age and sex significantly influence cTnI concentrations in healthy adult subjects, as demonstrated by means of a linear regression model between log-transformed cTnI concentration (dependent variable) and age and sex (independent variables) (Fig. 2 and Table 4). To better understand the relationship between cTnI concentrations and age in healthy adult subjects a spline regression analysis was performed. The spline regression plot indicates that cTnI concentrations of apparently healthy men and women are nearly constant from 18 to 55 years, and then they progressively increase by reaching on average about 3-fold higher cTnI values at 85 years (Fig. 3). Indeed, the 732 healthy subjects with age ≤ 55 years had significantly lower cTnI values than the 570 individuals with age > 55 years (2.7 ± 1.9 ng/L vs 4.1 ± 3.0 ng/L; $p < .0001$ by Wilcoxon/Kruskal-Wallis test). Mean, median, and percentile values of cTnI distribution values of the whole reference population, divided in age (i.e., ≤55 years vs > 55 years) and sex groups are also reported in Table 3. Of note, the values of distribution parameters for women and men with age > 55 year are not reported in Table 3 because the number of enrolled individuals was lower than 300, which is the needed number of case required by international guidelines for an accurate evaluation of 99th percentile URL values [2,15,16].

Table 3
Mean, median, and percentile values of cTnI distribution values (ng/L) of the reference population.

	Mean ± SD	Median	25th percentile	75th percentile	97.5th percentile	99th percentile	99th perc. BS* method (CI%)
Whole population (N = 1302)	3.3 ± 2.5	2.7	1.9	3.8	9.6	15.9	12.5 (11.8–14.2)* (11.7–14.7)** (11.3–16-7)***
Women (N = 650)	2.6 ± 1.8	2.2	1.6	3.2	6.5	13.6	8.9 (7.3–11.9)* (7.1–13.2)** (6.6–15.6)***
Men (N = 652)	3.8 ± 2.8	3.2	2.2	4.4	11.8	18.7	13.8 (12.4–16.7)* (12.3–18.0)** (11.8–25.8)***
WP ≤ 55 years (N = 732)	2.7 ± 1.9	2.3	1.6	3.2	8.0	14.8	11.0 (8.4–14.2)* (8.3–14.7)** (8.1–15.1)***
WP > 55 years (N = 570)	4.0 ± 2.8	3.3	2.3	4.7	11.8	18.5	13.7 (12.3–17.3)* (12.2–19.9)** (11.8–25.8)***
Women ≤ 55 years (N = 361)	2.1 ± 1.4	1.9	1.3	2.6	5.0	12.8	6.8 (5.1–11.9)* (5.0–14.0)** (4.8–16.8)***
Men ≤ 55 years (N = 370)	3.2 ± 2.1	2.8	2.0	3.7	9.1	15.7	12.1 (8.9–15.1)* (8.7–16.5)** (8.3–19.5)***

BS: Bootstrap method
* CI 90%.
** CI 95%.
*** CI 99%

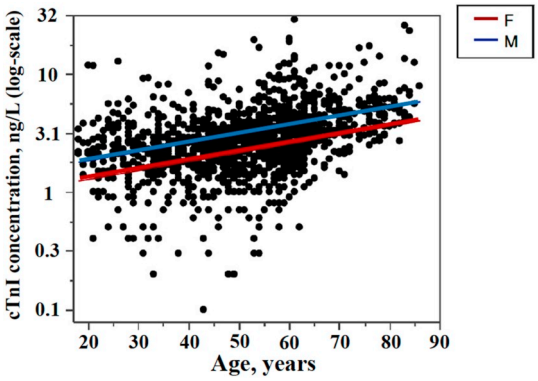

Fig. 2. Inter-relationship between log-cTnI concentration values (Y-axis) and age (X-axis). In the figure, the linear regressions with sex is also indicated (women: red color; men: blue color). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Regression analysis between cTnI values (dependent variable) and age and sex (independent variables).

R^2 = 0.227674 (P < .0001)
Intercept = 0.045 (SE 0.025) (P < .0001)
Age = 0.007 (SE 0.0005) (P < .0001)
Sex (F) = −0.082 (SE 0.007) (P < .0001)

SE: standard error

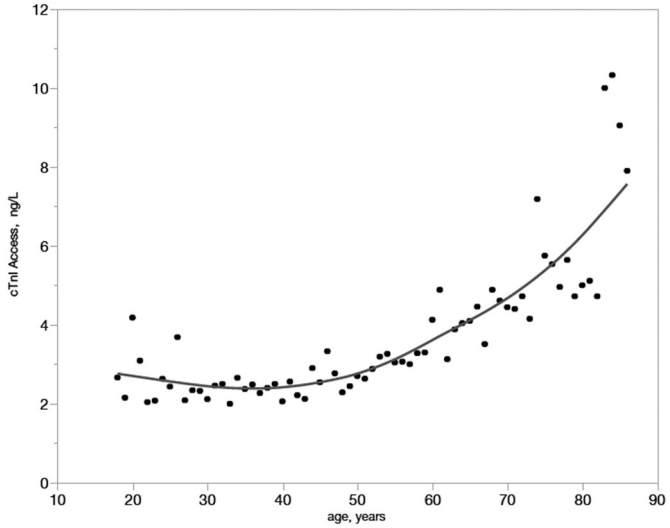

Fig. 3. The spline regression plot between cTnI concentrations (Y-axis) and age (X-axis) is reported in the figure. The spline regression analysis shows the trend between cTnI and age considering all the 1302 healthy subjects of the reference population. The curvilinear trend was fitted by considering the mean values of cTnI values of subjects with the same age interval.

4. Discussion

4.1. Analytical parameters and RCV of cTnI immunoassays

The results of the present study confirm that the Access cTnI method, using DxI platform, shows analytical characteristics and performances recommended by all international guidelines for high sensitivity methods for cTn assay (Table 1) [11,23,24]. To best of our

Table 5
Comparison of 99th percentile URL values in men and women.

	99th perc. women (CI%) (ng/L)	99th perc. men (CI%) (ng/L)	Mean ± SD and range of age (years)	Sample number (F/M)
Present study	13.6 (NP) 8.9 (BS) (7.3–11.9)* (7.1–13.2)**	18.7 (NP) 13.8 (BS) (12.4–16.7)* (12.3–18.0)**	F 51.7 ± 14,5; M 51.1 ± 13.9; Range 18–86	650/652
Pretorius et al. [23]	9.6 (7.3–16.3)*	20.9 (16.3–25.8)*	F 33.6; M 43.9; Range 18–80	647/1185
Manufacturer	11.6 (8.4–18.3)**	19.8 (14.0–42.9)**	Range 21–99	595/494

NP: nonparametric method; BS: bootstrap method.
* CI 90%.
** CI 95%.

knowledge, this study report for the first time the RCV and absolute critical change values of the Access cTnI method. The results reported in Table 2 can be useful for the diagnosis of acute coronary syndrome in patients presenting without persistent ST-segment elevation (NSTEMI), the detection of myocardial injury in asymptomatic patients, and also the evaluation of risk in the general population [1,3,4,26,27].

4.2. cTnI distribution values of reference population

The Fourth Universal Definition of Myocardial Infarction [3] states that: “the term myocardial injury should be used when there is evidence of elevated cardiac troponin values with at least one value above the 99th percentile URL”. Accordingly, the evaluation of 99th percentile URL value of cTnI and cTnT has a fundamental relevance in the diagnosis of all cardiac diseases. Unfortunately, the accurate evaluation of the 99th percentile value in the reference population presents some important limitations, related to analytical performance of cTn methods, demographic characteristic of reference populations, and statistical approach used for calculation of distribution parameters [15,16,28]. Indeed, large variations of 99th percentile URL values are reported in the literature even for the same cTnI or cTnT immunoassay method [15,16].

The results of the present study confirm that age and sex can strongly affect the measurement of 99th percentile value, even when the criteria for selection of reference population are well in accordance with recommendations made by international guidelines [2,15]. In particular, the results of the present study (as reported in Fig. 3) demonstrate that after the age of 55 years there is in both men and women a progressive rise in cTnI concentrations, even when the healthy status of the individuals enrolled in the study are well characterized using a thorough clinical history together with laboratory (including assay of BNP/NT-proBNP) and an accurate cardiac investigation (also including ECG and echocardiography).

Sex-related 99th percentile URL values observed in this study, reported by Pretorius et al. [24] and suggested by the manufacturer are reported in Table 5. The results observed in the present study are well in accordance with literature data because the 99th percentile URL values of present study are included within the confidence interval reported by Pretorius et al. [24] and manufacturer technical bulletin (Table 5). The between-study bias of 99th URL values are probably due to differences in age ranges, sample sizes, and sex-ratios of enrolled healthy subjects.

The results of the present study are well in agreement with previous results [28,29], indicating that both calculation of 99th percentile and method to detect outliers can greatly affect the 99th percentile URL of cTnI high-sensitivity methods. In this study both nonparametric bootstrap approach and “Tukey's fences” method were used to reduce the effect of outliers [22]. Indeed, the nonparametric bootstrap method allowed significantly lower 99th percentile values than nonparametric one for both whole population and subgroups related to age and sex (mean ± SD: 11.3 ± 2.6 ng/L vs 15.7 ± 2.3 ng/L;$N = 7$, $P < .0001$) (Table 3). However, all 99th percentile values calculated with nonparametric approach are within the 99% CI of those obtained with nonparametric bootstrap method (Table 3). From a clinical point of view, the use in the clinical practice of 99th percentile URL values, calculated with nonparametric bootstrap method should theoretically allow a better sensitivity but also a worse specificity both in detection of myocardial injury and diagnosis of myocardial infarction [3]. Of course, the relative cost/benefit of these two statistical approaches for the calculation of the 99th percentile URL value of the Access cTnI method should be evaluated by specifically designed clinical studies.

As previously reported in detail [11], the Access cTnI method satisfies the first fundamental criterium for high-sensitivity method for cTnI assay. Indeed, the Access cTnI method measures the 99th percentile URL with an imprecision less than CV 10% (Table 1 and Fig. 1), as requested by international guidelines [2]. The results, reported in the present study, demonstrate that the Access cTnI method satisfies also the second criterium for high-sensitivity method because it measures cTn concentrations above the LoD in more than in 50% healthy subjects of both men and women [2].

4.3. Strengths and limitations of the study

The strengths of this study are related to the experimental design. Several Authors emphasized the need of both characterized reference populations and standardized statistical approach to calculate cTnI 99th percentiles [2,15,16,28,29]. Our study is a multi-center study which enrolled a well selected healthy reference population according to indications made by international guidelines and expert documents [2,15]. The study reference population consisted of 1302 healthy adults subjects with a large age range (18 to 86 years), and including 650 women and 652 men, well matched for age and sex-ratio. Furthermore, for the calculation of 99th percentile URL values, the standard nonparametric approach, as suggested by international guidelines [2], and also the more complex, but also more accurate nonparametric bootstrap method were used [22].

A relative limitation of this study is the low number of healthy subjects with age > 55 years enrolled in this study (i.e., 570). This number did not allow an accurate evaluation of the 99th percentile URL values divided for men and women with age > 55 years. However, it is well known that it is very difficult to enroll a great number of well characterized healthy subjects with age > 70 years [15,16].

5. Conclusions

The results of the present study confirm that the Access hsTnI method using the UniCell DxI 800 platform satisfies all the criteria and quality specifications required by the most recent international guidelines for high-sensitivity methods for cTn assay [2]. Furthermore, the results of this study confirm that the calculation of the 99th percentile URL values are greatly affected not only by age and sex of the reference population, but also by the statistical approach used for calculation of cTnI distribution parameters.

Acknowledgements

All the immunoassay kits used in this study were kindly supported by the manufacturers, namely: Beckman Coulter S.P.A. Italy (Cassina de' Pecchi, Milan, Italy).

References

[1] M. Roffi, C. Patrono, J.P. Collet, C. Mueller, M. Valgimigli, F. Andreotti, J.J. Bax, M.A. Borger, C. Brotons, D.P. Chew, B. Gencer, G. Hasenfuss, K. Kjeldsen, P. Lancellotti, U. Landmesser, J. Mehilli, D. Mukherjee, R.F. Storey, S. Windecker,

2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC), Eur. Heart J. 37 (2016) 267–315.
[2] A.H.B. Wu, R.H. Christenson, D.N. Greene, A.S. Jaffe, P.A. Kavsak, J. Ordonez-Lianos, F.S. Apple, Clinical laboratory practice recommendations for the use of cardiac troponin in acute coronary syndrome: expert opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-markers of the International Federation of Clinical Chemistry and Laboratory Medicine, Clin. Chem. 64 (2018) 645–655.
[3] K. Thygesen, J.S. Alpert, A.S. Jaffe, B.R. Chaitman, J.J. Bax, D.A. Morrow, D.A. White, Fourth universal definition of myocardial infarction, J. Am. Coll. Cardiol. 72 (2018) 2231–2264.
[4] A. Clerico, A. Fortunato, A. Ripoli, C. Prontera, G.C. Zucchelli, M. Emdin, Distribution of plasma cardiac troponin I values in healthy subjects: pathophysiological considerations, Clin. Chem. Lab. Med. 46 (2008) 804–808.
[5] A. Giannoni, S. Giovannini, A. Clerico, Measurement of circulating concentrations of cardiac troponin I and T in healthy subjects: a tool for monitoring myocardial tissue renewal? Clin. Chem. Lab. Med. 47 (2009) 1167–1177.
[6] C. Prontera, A. Fortunato, S. Storti, A. Mercuri, G. Longombardo, G.C. Zucchelli, M. Emdin, A. Clerico, Evaluation of analytical performance of the Siemens ADVIA TnI ultra immunoassay, Clin. Chem. 53 (2007) 1722–1723.
[7] A.K. Saenger, R. Beyrau, S. Braun, R. Cooray, A. Dolci, H. Freidank, E. Giannitsis, S. Gustafson, B. Handy, H. Katus, S.E. Melanson, M. Panteghini, P. Venge, M. Zorn, P. Jarolim, D. Bruton, J. Jarausch, A.S. Jaffe, Multicentre analytical evaluation of a high-sensitive troponin T assay, Clin. Chim. Acta 412 (2011) 748–754.
[8] P. Venge, S. James, L. Jansson, B. Lindahl, Clinical performance of two highly sensitive cardiac troponin I assays, Clin. Chem. 55 (2009) 109–116.
[9] M. Krintus, M. Kozinsk, P. Boudry, N.E. Capell, U. Köller, K. Lackner, European multicenter analytical evaluation of the Abbott ARCHITECT STAT high sensitive troponin I immunoassay, Clin. Chem. Lab. Med. 52 (2016) 1657–1665.
[10] C. Caselli, G. Cangemi, S. Masotti, R. Ragusa, I. Gennai, S. Del Ry, C. Prontera, A. Clerico, Plasma cardiac troponin I concentrations in healthy neonates, children and adolescents measured with a high sensitive immunoassay method: high sensitive troponin I in pediatric age, Clin. Chim. Acta 458 (2016) 68–71.
[11] S. Masotti, C. Prontera, V. Musetti, S. Storti, R. Ndreu, G.C. Zucchelli, C. Passino, A. Clerico, Evaluation of analytical performance of a new high-sensitivity immunoassay for cardiac troponin I, Clin. Chem. Lab. Med. 56 (2018) 492–501.
[12] S. Masotti, V. Musetti, C. Prontera, S. Storti, C. Passino, G.C. Zucchelli, A. Clerico, Evaluation of analytical performance of a chemiluminescence enzyme immunoassay (CLEIA) for cTnI using the automated AIA-CL2400 platform, Clin. Chem. Lab. Med. 56 (2018) e174–e176.
[13] V. Musetti, S. Masotti, C. Prontera, S. Storti, R. Ndreu, G.C. Zucchelli, C. Passino, M. Emdin, A. Clerico, Evaluation of the analytical performance of a new ADVIA immunoassay using the Centaur XPT platform system for the measurement of cardiac troponin I, Clin. Chem. Lab. Med. 56 (2018) e229–e231.
[14] J. Marjot, T.E. Kaier, E.D. Martin, S.S. Reji, O. Copeland, M. Iqbal, B. Goodson, S. Hamren, S.E. Harding, M.S. Marber, Quantifying the release of biomarkers of myocardial necrosis from cardiac myocytes and intact myocardium, Clin. Chem. 63 (2017) 990–996.
[15] Y. Sandoval, F.S. Apple, The global need to define normality: the 99th percentile value of cardiac troponin, Clin. Chem. 60 (2014) 455–462.
[16] A. Clerico, M. Zanonotto, M. Ripoli, S. Masotti, C. Prontera, C. Passino, M. Plebani, The 99th percentile of reference population for cTnI and cTnT assay: methodology, pathophysiology, and clinical implications, Clin. Chem. Lab. Med. 55 (2017) 1634–1651.
[17] M. Franzini, V. Lorenzoni, S. Masotti, C. Prontera, D. Chiappino, D. Della Latta, M. Dayes, I. Deluggi, M. Zuin, L. Ferrigno, A. Mele, F. Marcucci, C.A. Caserta, P. Surace, A. Messineo, G. Turchetti, C. Passino, M. Emdin, A. Clerico, The calculation of the cardiac troponin T 99th percentile of the reference population is affected by age, gender, and population selection: a multicenter study in Italy, Clin. Chim. Acta 438 (2015) 376–381.
[18] CLSI EP17-A Protocol, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guidelines, Vol. 24 (34) (2004) (Wayne, Pennsylvania).
[19] CLSI EP5-A2 Protocol, Evaluation of Precision Performance of quantitative Measurement Methods; Approved Guideline, Second edition, Vol. 24(25) (2004) (Wayne, Pennsylvania).
[20] C.G. Fraser, Reference change values, Clin. Chem. Lab. Med. 50 (2011) 807–812.
[21] W. Tukey, Exploratory Data Analysis, Addison-Wesley, Reading (PA), 1977.
[22] J. Carpenter, J. Bithell, Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians, Stat. Med. 19 (2000) 1141–1164.
[23] G. Lippi, A. Ferrari, G. Gandini, M. Gelagi, C. Lo Cascio, G.L. Salvagno, Analytical evaluation of the new Beckman Coulter Access high sensitivity cardiac troponin I, Clin. Chem. Lab. 56 (2017) 157–161.
[24] C.J. Pretorius, R.J. Tate, U. Wilgen, L. Cullen, J.P.J. Ungerer, A critical evaluation of the Beckman Coulter Access hsTnI: Analytical performance, reference interval and concordance, Clin. Biochem. 55 (2018) 49–55.
[25] N. van der Linden, J.M. Hilderink, T. Cornelis, D.M. Kimenai, L.J.J. Klinkenberg, W.P. van Doorn, E.J.R. Litjens, J.D.E. van Suijlen, L.J.C. van Loon, O. Beckers, J.P. Kooman, S.J.R. Meex, Twenty-four-hour biological variation profiles of cardiac troponin I in individuals with or without chronic kidney disease, Clin. Chem. 63 (2017) 1655–1656.
[26] J. Mair, B. Lindahl, O. Hammarsten, C. Müller, E. Giannitsis, K. Huber, M. Möckel, M. Plebani, K. Thygesen, A.S. Jaffe, How is cardiac troponin released from injured myocardium? Eur. Heart J. Acute Cardiovasc. Care 7 (2018) 553–560.
[27] K.M. Eggers, P. Venge, B. Lindhal, L. Lind, Cardiac troponin I levels measured with a high-sensitive assay increase over time and are strong predictors of mortality in an elderly population, J. Am. Coll. Cardiol. 61 (2013) 1906–1913.
[28] K.M. Eggers, F.S. Apple, L. Lind, B. Lindhal, The applied statistical approach highly influences the 99th percentile of cardiac troponin I, Clin. Biochem. 49 (2016) 1109–1112.
[29] P.E. Hickman, G. Koerbin, J.M. Potter, W.P. Abhayaratna, Statistical considerations for determining high-sensitivity cardiac troponin reference intervals, Clin. Biochem. 50 (2017) 502–505.